

# Effect of the 5-*HTTLPR* Genotype on Memory Performance in Older Adults

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I declare that this report is my own original work and that contributions of others have been duly acknowledged.

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# **Effect of the 5-*HTTLPR* Genotype on Memory Performance in Older Adults**

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### **Abstract**

The s allele, of the *5-HTTLPR* genotype has been associated with a negative effect on memory function, however very few studies have examined the impact of this gene polymorphism in older age. This is important given the functional impact of memory decline in ageing, and given that serotonin declines with age. The present study examined the effect of the *5-HTTLPR* genotype (s/s and s/l versus l/l genotypes) on episodic and working memory function across age. This effect was also examined separately across males and females. A total of 298 female and 140 male participants were recruited as part of The Healthy Brain Project with a mean age 60.35 years (range 49-80). Participants were administered tests of visual and verbal episodic and working memory as well as being genotyped for the *5-HTTLPR* gene. Moderation analysis revealed decline in verbal episodic memory across age was differentially predicted by the *5-HTTLPR* genotype, with this effect being moderated by sex. There was no significant moderation of *5-HTTLPR* on visual episodic or working memory measures across age. These findings suggest serotonin influences verbal episodic memory specifically and has a differential effect in women and men across age.

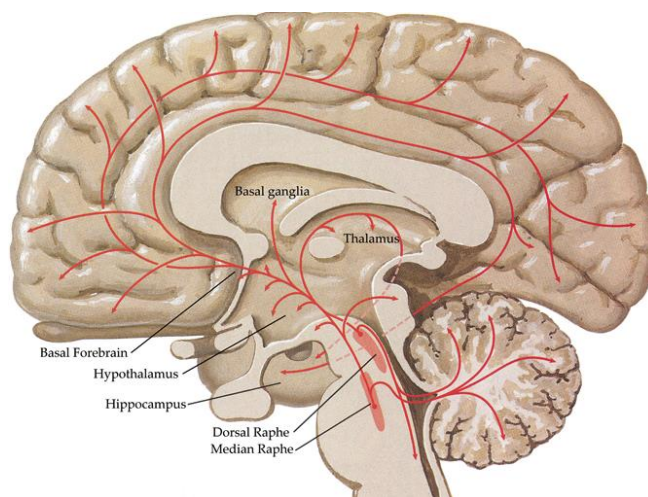
Serotonin (5-Hydroxytryptamine; 5-HT) is a key neurotransmitter in the CNS and its impact on neurocognition is well established (Seyedabadi, Fakhfouri, Ramezani, Mehr, & Rahimian, 2014). There is evidence for a relationship between 5-HT and memory performance, however very few studies have examined the role of serotonin on memory decline in ageing. This is important to investigate, as ageing is associated with a reduction in levels of circulating serotonin as well as a decline in memory functions (Rodriguez, Noristani, & Verkhatsky, 2012). Memory impairments in ageing can lead to significant functional deficits and are a major contributor to lowered quality of life for ageing individuals (Millan-Calenti et al., 2012). While memory impairments have been associated with dysfunctions of the serotonin system (Mendelsohn, Riedel, & Sambeth, 2009), the relationship between markers of 5-HT function such as the serotonin transporter (5-HTT), have had limited investigation in conjunction with ageing. Functional polymorphisms of the 5-*HTT* gene are associated with differential transcription efficiency of 5-HTT (Lesch et al., 1996). Therefore these polymorphisms provide a promising marker for examining the impact of serotonin regulation on rates of memory decline across age. To further explore the impact of serotonin on memory in the ageing brain, the aim of the current study was to examine the effect of functional polymorphisms of the 5-*HTT* gene on episodic and working memory performance in older adults.

### **Serotonin and the brain**

Serotonin is widely dispersed throughout the brain and is involved in many behaviours such as feeding, sleeping, sexual behaviour, mood and cognition (Lucki, 1998). The raphe nuclei of the brainstem is where the cell bodies of 5-HT neurons originate and is therefore the site where 5-HT is produced and stored (Jacobs & Azmitia, 1992). Ascending 5-HT neurons from the raphe nuclei project to multiple

cortical and subcortical brain regions, innervating many brain areas including those involved in memory, such as the pre-frontal cortex and hippocampus (Mattson, Maudsley, & Martin, 2004; Struder & Weicker, 2001: Refer to Figure 1).

Synthesis of 5-HT depends on the availability of its amino acid precursor, L-tryptophan which is derived from diet and converted to 5-HT after crossing the blood brain barrier (Vergne & Nemeroff, 2006). The activity of serotonin in the brain is determined by the serotonin transporter and several different 5-HT receptors (5-HT<sub>1</sub>-5-HT<sub>7</sub>) (Hannon & Hoyer, 2008).



*Figure 1.* Projections of serotonergic neurons in the central nervous system from the raphe nucleus to multiple cortical regions including the hippocampus and pre-frontal cortex, involved in memory performance.

### **Serotonin and Memory**

Serotonin is thought to play a critical role in both working memory (WM) and episodic memory through innervations of 5-HT neurons to the prefrontal cortex, hippocampus and entorhinal cortex, regions of the brain implicated in memory function (Jacobs & Azmitia, 1992; Meneses & Liy-Salmeron, 2012). Memory is the term used to describe neural structures and processes involved in the storage of

information and its retrieval (Lezak, Howieson, Bigler, Tranel, 2012). Memory can be broadly classified as either short-term (working memory) or long-term memory. WM employs different neural processes to LTM and allows information to be briefly stored and manipulated via temporary potentiation of neural circuits (Sreenivasan, Curtis, & D'Esposito, 2014).

Long-term memory, on the other hand, can be divided into declarative and procedural types (Barros, Tufik, & Andersen, 2015). Episodic memory is a division of declarative memory and involves the conscious recollection of facts and events, involving three phases (encoding, consolidation, retrieval: Pacheco et al., 2009). Episodic memory is associated with activation of the hippocampus and is dependent on hippocampal connections with parahippocampal regions and the neocortex (Bearden et al., 2012; Tulving & Markowitsch, 1998)

While it is established that 5-HT is associated with neurocognition, of all monoamines, serotonin's role in memory is thought to be the least understood (Pacheco et al., 2012). Increasing evidence for the direct participation of 5-HT in memory comes from experimental manipulations of tryptophan availability (Meneses & Liy-Salmeron, 2012).

### **Acute Tryptophan Depletion and Memory**

Increasing the availability of L-tryptophan, which is associated with increased levels of serotonin, has been found to increase memory ability in healthy adults, elderly adults (Porter et al., 2005) as well as in Alzheimer's disease (AD) patients (Porter et al., 2003). Further evidence for the role of serotonin in memory comes from depletion of 5-HT availability commonly achieved through acute tryptophan depletion (ATD) (Mendelsohn et al., 2009). Reducing brain 5-HT via ATD has been associated with impaired memory function (Helmbold et al., 2013;

Porter et al., 2005, Merens et al., 2008). The key findings from ATD studies reveal an impairing effect on verbal episodic memory but not verbal WM or visual WM (For a summary table of relevant ATD studies, see Appendix A)

While there is support for the negative effects of reduced serotonin on episodic memory, some studies have also revealed no effect (Hayward, Goodwin, Cowen, & Harmer, 2005; Hughes et al., 2003). This could be due to the use of small sample sizes leading to underpowering of studies, or may be due to the bulk of studies examining healthy younger adults. As older adults have reduced levels of serotonin compared to younger adults (Rodriguez et al., 2012), the effects of ATD for younger adults may differ to those for older adults. Furthermore, inconsistent findings could be accounted for by the different dosages of ATD used between studies as it has been found that high versus low dosages of ATD have differential effects on cognitive function (Merens et al. 2011).

In regards to WM, most studies suggest ATD does not impair WM in younger adults (Harrison et al., 2004; Luciana, Burgund, Berman, & Hanson, 2001; Stewart, Deary, & Ebmeier, 2002). However, in older adults, several reports of reduced WM after ATD have been made (Mace et al., 2011). In addition, Mace, Porter, O'Brien, and Gallagher (2008), found that ATD in high doses impaired WM performance in females but not males. These findings are likely due to the decrease in WM performance accompanied by ageing, however it could also be due to the fact that serotonin levels are lower in females compared to males (Nishizawa et al., 1997) and in older adults compared to younger adults (Rodriguez et al., 2012). By having reduced levels of 5-HT it may increase the sensitivity of females and older adults to alterations 5-HT system functioning. In support of this conclusion, it has been

documented that the detrimental effects of ATD on verbal memory functions are more apparent in females than males (Sambeth et al., 2007).

### **The Impact of Ageing on Serotonin and Memory**

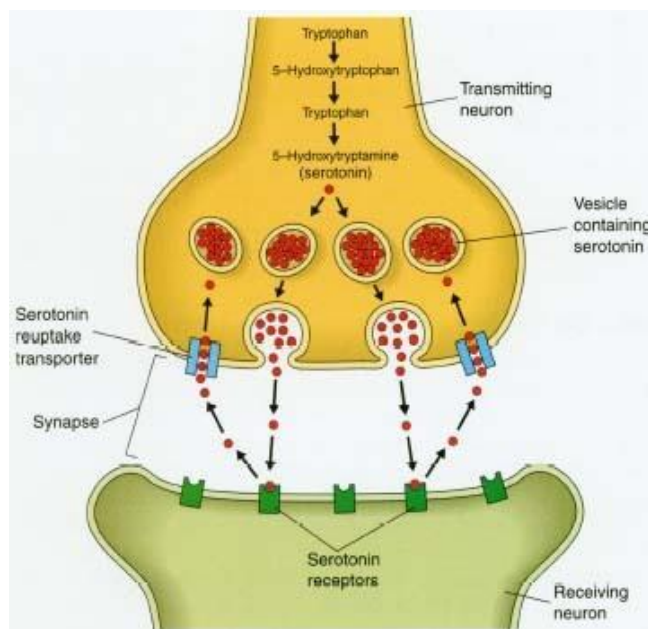
The most affected domains of cognition that decline with age are memory and attention (Gilsky, 2007). Areas of the brain responsible for these functions, such as the pre-frontal cortex and the hippocampus, have a rich supply of 5-HT neuron activity (Meneses & Liy-Salmeron, 2012). These areas experience reduced serotonin secretion across age resulting in a general decline in levels of circulating serotonin (Rodriguez et al.). In conjunction with decreased serotonin levels across age, WM and episodic memory performance both decrease with age (Mendelsohn et al., 2009). Therefore, the potential association between markers of serotonin function and memory may be more evident in ageing-individuals.

### **Sex differences in Serotonin Levels**

In addition to a decrease in circulating levels of 5-HT across age, females compared to males have approximately 50 percent lower levels of circulating serotonin in the central nervous system (Nishizawa et al., 1997). Therefore females may be more prone to neurocognitive deficits that may occur from a dysfunction in the 5-HT system. This is somewhat supported by the higher prevalence for mood and anxiety disorders, associated with dysfunction of the serotonin system, seen in females compared to males (Helmbold et al., 2013; Kessler et al., 2005). The already explained sensitivity of females to manipulation of 5-HT levels via ATD, highlights that females may be more susceptible to memory dysfunction in older age when there is a reduction in 5-HT (Helmbold et al., 2013; Sambeth et al., 2007). One way to examine the impact of serotonin on memory is via the serotonin transporter gene (5-HTT).

## The Serotonin Transporter

A key regulator of 5-HT function is the 5-HTT, a membrane bound protein that is responsible for the re-uptake of 5-HT from the synaptic cleft back into the presynaptic neuron (Lesch, Wolozin, Murphy, & Reiderer, 1993: see Figure 2). Its function determines the duration and intensity of the serotonin signal at the synapse. 5-HTT's are distributed in brain areas associated with memory function with the highest levels found in the raphe nuclei and subcortical structures, followed by the hippocampus and the prefrontal cortex (Madsen et al., 2011; Murphy, Lerner, Rudnick, & Lesch, 2004).

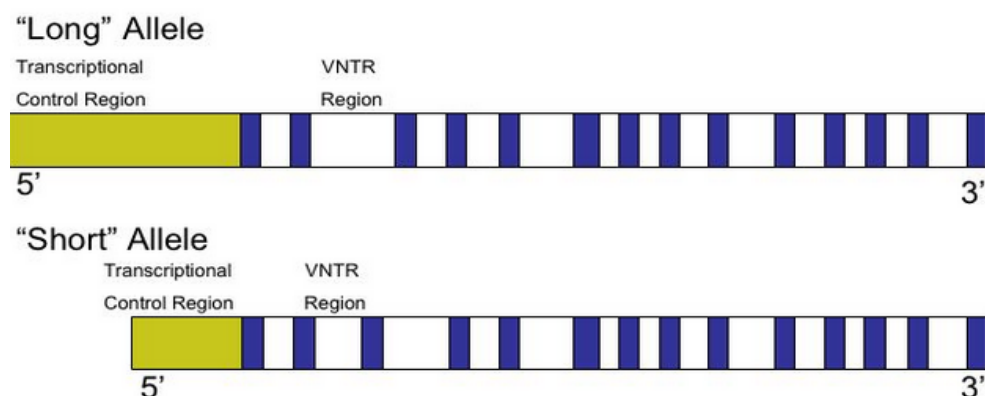


*Figure 2.* Depiction of 5-HTT action at the synapse, regulating amount of 5-HT in the synaptic cleft.

The gene that codes for the 5-HTT (*SLC6A4*) is located on chromosome 17 (Lesch et al., 1993). The promoter region of this gene has a functional polymorphism (serotonin transporter linked-polymorphic region: *5-HTTLPR*) that results in two allelic variants consisting of a 44-base pair insertion (long “l” allele) or deletion (short “s” allele: see Figure 3). The presence of the s allele is associated with

reduced transcription of the *5-HTT* gene resulting in a 40 per cent reduction in density of 5-HTT sites and binding of 5-HT to receptors in comparison to the long allele (Greenberg et al., 1999). The reduced 5-HTT sites in s allele carriers results in a two-fold decrease in 5-HT uptake at the synapse and therefore less modulation of 5-HT (Lesch et al., 1996).

While reduced re-uptake results in greater synaptic 5-HT, it is suggested that, over time, this results in lower 5-HT signaling at the post synaptic neuron, which could be due to neuronal changes such as lower receptor sensitivity (Williams et al., 2003). As this transporter is the key regulator of serotonin function, the polymorphism variants of the *5-HTTLPR* gene that code for the transcription of 5-HTT may be a promising marker for detecting accelerated memory decline across age.



*Figure 3.* Depiction of the short and long alleles of the serotonin transporter gene that result from a polymorphism in the promoter region of *SLC6A4*

### ***5-HTTLPR* Effect on Hippocampus Architecture**

The *5-HTTLPR* genotype not only impacts the expression of the 5-HTT and concentrations of circulating 5-HT, the polymorphism variants have been associated



with changes in the brain's architecture (Price et al., 2013). Associations between, 5-*HTTLPR* genotype and neurodegeneration in the hippocampus, as well as other brain areas, have been demonstrated through *SLC6A4* knockout mice (Dannlowski et al., 2014; Olivier et al., 2008). More specifically, s allele carriers have been associated with reduced grey matter in the hippocampus (Olivier et al., 2008) and areas of the prefrontal cortex (Frodl et al., 2008; Pezawas et al., 2005), compared to individuals homozygous for the long genotype. Based on these findings, lower memory performance associated with the s allele compared to the l-homozygous genotype might be expected.

In a healthy adult sample, Price et al. (2013) also associated the s allele with smaller hippocampal volume, however for males the association was only present for those who had experienced significant childhood adversity. Erker et al. (2011) revealed that individuals with depression and the s/s genotype had smaller hippocampal volumes than depressed individuals with the l/l genotype. On the basis of these findings, it could be argued that the association between the s allele and lower grey matter volume is moderated by stress on the serotonergic system, for example, traumatic events or depression, which have both been found to impact on the serotonin system (Dannlowski et al., 2014; Firk & Markus, 2007; Frodl et al., 2015; Pezawas et al., 2005).

### **Empirical Evidence: 5-*HTTLPR* and Memory**

In addition to the findings of ATD effects on memory function and the association between the 5-*HTTLPR* genotype variants and hippocampal volume, there is research examining the impact of 5-*HTTLPR* genotypes on memory function. However, findings have been somewhat inconsistent (see Table 1), with some studies providing support for the s allele as a risk allele for memory

dysfunction, whilst others report the opposite or no association. One possible source of this inconsistency in findings is that many studies have small sample sizes ( $n < 100$ ) and are thus underpowered for examining genotype effects. However, even in studies with larger sample sizes, a further potential source of inconsistency is that the *5-HTTLPR* genotype may have a specific effect on particular types of memory.

### **Episodic memory**

O'Hara et al. (2007), examined the interaction of the *5-HTTLPR* genotype, cumulative life stress and cortisol levels on cognitive performance of 154 older adults (60-100 years of age). In their study, an association between the *5-HTTLPR* genotype and lower delayed recall scores on verbal episodic memory was found, with s allele carriers showing worse memory performance compared to l-homozygous individuals. The s allele has also been associated with poorer visual episodic memory performance compared to l-homozygous genotypes (Price et al., 2013).

In contrast, a study conducted by Payton et al. (2005) examining the effect of this genotype on cognitive abilities in 758 older adults (65-85 years of age) did not find any associations between the *5-HTTLPR* genotype and episodic memory. Payton et al., did, however, show that an additional polymorphism associated with the *SLC6A4* gene, namely VNTR2 was associated with rate of cognitive decline. It should be noted here that the memory tests utilised by Payton and colleagues, were not standard tests. Diverging from the aforementioned findings, another study that examined older adults (sample size of 23) associated the s allele with deficits in source memory monitoring (Pacheco et al., 2012).

## Working memory

Contrary to the s allele being identified as a risk allele for lower episodic memory performance, there are some findings that suggest the s allele of the 5-*HTTLPR* genotype is associated with better WM performance. For example, Enge et al. (2011) demonstrated both males and females (18-33 years old) homozygous for the s genotype had better WM performance on an n-back task than individuals with the s/l or l/l genotypes. Anderson, Bell and Awh (2010) observed similar results, whereby carriers of the short allele (s/s and s/l genotype) were associated with better visual WM performance as measured by a visual change detection task.

Contrary to the beneficial effects reported for the s allele and WM, updating and monitoring of WM was found to be worse for the s/s genotype compared to the s/l and l/l genotype in healthy women (Weiss et al., 2014) suggesting there may be sex specific effects of 5-*HTTLPR* genotype and WM. Furthermore, there are some reports of null findings for associations between WM and the 5-*HTT* genotypes (Payton et al., 2005; Reneman et al., 2006). As outlined in the literature review summarised in Table 1, the effects of the 5-*HTTLPR* genotype variants on episodic memory and WM are inconsistent. Possible explanations for these inconsistencies are low power, due to small sample sizes, variable age ranges used and not controlling for or taking into account sex. In addition, ethnic heterogeneity can be responsible for different findings across studies due to differential serotonin turnover rates (Goldman, Glei, Lin, & Weinstein, 2010). For example African Americans have increased turnover rates compared to Caucasians (Price et al., 2013). Despite the identified role of 5-HT in memory the association between the 5-*HTTLPR* genotype and memory function remains unclear.

### Selective Effect of the *5-HTTLPR* Genotype

In addition to the inconsistent findings of the *5-HTTLPR* polymorphism variants and WM, there is some evidence for 5-HT having a selectively modulating effect on verbal memory functions (Helmbold et al., 2013; Price et al., 2013; Zilles et al., 2012). In conjunction with the neuroanatomical separation of Verbal and spatial WM components, Zilles et al., (2012) provide evidence that the *5-HTTLPR* genotype has a selective effect on functions of verbal WM. In their study, l-homozygosity was associated with better verbal WM performance than s allele carriers (s/s and s/l) whereas there was no difference found between genotype groups for spatial WM performance. However, there have been limited replications of these findings and the small sample size of only 20 healthy participants means replication is warranted. Despite the limitations of the Zilles et al study, a systematic review of ATD effects on memory also revealed that changes in 5-HT most robustly affects verbal episodic memory (Mendelsohn et al., 2009). Furthermore Helmbold et al., revealed verbal episodic memory performance was vulnerable to impairment during ATD. It is therefore expected that any difference between the *5-HTTLPR* genotypes on memory performance will be greater for measures of verbal memory compared to visual memory.

Table 1

*Summary of Findings: Effect of 5-HTTLPR Genotype on Memory Performance*

<b>Study</b>	<b>Participants (age)</b>	<b>Memory measures</b>	<b>Findings</b>
Payton et al. (2005)	750 healthy elderly (50-85)	Immediate and Delayed Verbal recall	No association between 5-HTTLPR variants and memory scores.
O'Hara et al. (2007)	154 healthy elderly (60-100)	Rey Auditory Verbal Learning Test	s/s and s/l genotypes associated with significantly worse delayed recall than l/l genotype
Anderson et al. (2010)	86 healthy participants (18-35)	Change detection task (visual WM).	s/s and s/l groups performed better in WM measures than l/l group.
Enge et al. (2011)	130 participants (18-33)	n-back task (WM).	s carriers show significantly more efficient executive control of WM.
Zilles et al. (2012)	20 healthy + 80 psychiatric patients	Verbal and visuospatial WM rehearsal WM task	l/l individuals performed better than s-carriers on verbal WM
Pacheco et al. (2012)	17 young adults (19-30) 23 elderly adults (60-81)	Memory monitoring: Source recognition task	Specific deficits in memory monitoring for s/s and s/l genotypes for older adults.
Price et al. (2013)	51 young adults (18-25)	California Verbal Learning + Rey Complex Figure Test	Female s allele carriers had poorer visual recall compared to female l carriers. No relationship found for males.
Weiss et al. (2014)	255 healthy women (18-59)	Mittenecker pointing test	Poorer performance for s/s compared to s/l and l/l on updating WM.

### **Serotonin effect in a stressed system**

The majority of genetic effects on neural function are polygenetic and may have only small impacts on behavioural phenotypes (Franks, 2014; Plomin & McGuffin, 2003). In this regard, it is difficult to establish a direct relationship between a specific genotype and a behavioural trait. However, examination of the serotonin system under stress is proposed to give greater insight into the relationship between a candidate gene and a behavioural trait such as memory. The importance of the gene X environment interaction in examining the effect of the *5-HTTLPR* genotype has been demonstrated in many published reports, whereby the s allele of the *5-HTTLPR* genotype has been suggested to predispose individuals to developing major depression following significant life adversity (Daniele et al., 2011; Juhasz, 2015). For example, increased diagnosis of depression and anxiety (Stein, Schork, & Gelernter, 2008) has been found for s allele carriers compared to l/l genotypes in adolescents (Eley et al., 2004) and adults (Kendler, Kuhn, Vittum, Prescott, & Riley, 2005) when faced with stressful life events, which also challenge brain serotonin levels (Firk & Markus 2007).

When investigating *5-HTTLPR* genotype effects on verbal memory under ATD conditions, Roiser, Muller, Clark, and Sahakian (2007), found s-carriers had poorer verbal recall when under ATD conditions compared to control participants. Furthermore there was no effect between control and ATD conditions for l/l homozygous participants. These findings indicate that the s allele can be considered a risk allele for reduced verbal memory performance when the serotonergic system is compromised, for example, in ATD conditions. One way to examine the serotonin system when compromised is to examine the impact of serotonin on memory in the

ageing brain, as ageing is associated with significant reductions in serotonin. A second way is to examine the impact of serotonin on memory in females.

### **Effect of 5-HTTLPR genotype in females**

Differences in the rate of 5-HT metabolism between males and females have been suggested to result in sex moderating the effect of *5-HTTLPR* on indicators of 5-HT function including memory (Helmbold et al., 2013; Price et al., 2013). As highlighted previously, females have approximately 50 percent lower levels of circulating 5-HT than males (Nishizawa et al., 1997). Brummett et al., (2008) found that the effect of the *5-HTTLPR* genotype on depression was moderated by sex in addition to stressful life events. More specifically the l allele was associated with higher levels of depression for males when accompanied with a high stressor, whereas for females the s allele was associated with increased depressive symptoms in conjunction with a stressor. Therefore, it might be predicted that a greater negative effect of the s allele on memory will be observed in females.

### **Effect of serotonin in older age**

Another exemplar where the serotonin system is under stress is old age. As age increases, levels of centrally circulating serotonin progressively decreases resulting in stress on the serotonergic system (Payton et al., 2005). In addition to the moderation of stressful life events on the relationship between the s allele and neuropsychiatric disorders, it has been suggested that the *5-HTTLPR* genotype may interact with the normal ageing process. For example, in a study conducted by Li et al. (1997), the short allele was found to be elevated in AD patients compared to control participants. In addition Marini et al (2011) described an increased frequency of the short allele in subjects with mild cognitive impairment. It has been suggested that there is a differential effect of the *5-HTTLPR* genotype on older adults whereby

the short allele may decrease older adults ability to adapt to changes in serotonin function (O'Hara et al., 2007), this could result in behavioural symptoms, such as memory impairments. Therefore, as serotonin is related to memory function, the s allele of the *5-HTTLPR* genotype may be associated with memory impairments more so than the long allele in older adults.

In summary, there is increasing evidence that dysfunctions in the serotonin system affect memory functioning, particularly verbal memory. As the 5-HTT is a key regulator of serotonin function, the *5-HTTLPR* genotype variants are a promising marker to investigate for differential effects on memory function. There are inconsistencies in the literature on the differential effects of the *5-HTTLPR* genotypes on episodic and WM, which may be the result of small effect sizes, underpowered studies, not examining sex specificity, and a focus on healthy young controls. As there are clear effects of this genotype on hippocampal volume and clinical disorders when accompanied by environmental stressors, it is predicted that the inclusion of internal stressors on the serotonin system such as age and sex, which are associated with a decreased level of 5-HT, may reveal a differential effect of the *5-HTTLPR* genotype on memory function if there is one.

### **Aim and hypotheses**

The aim of the current study was to better clarify the possible role of *5-HTTLPR* genotype variants on memory in an elderly population. As there is a general decrease in serotonin across age, it was predicted that in an elderly population the additional stress on the serotonin system would increase the likelihood of detecting effects of *5-HTTLPR* genotype on memory function.

- 1) It was hypothesised that the relationship between age and relative memory function would be moderated by the *5-HTTLPR* genotype. More specifically



it was predicted that s carriers (s/s and s/l genotypes) would be associated with a stronger negative relationship between age and memory performance as compared to the l/l genotype.

- 2) In accordance with the findings that altered 5-HT levels in the CNS most robustly affect verbal memory it was hypothesised that the proposed differential effects between s carriers and l-homozygous individuals would be more pronounced for verbal memory as determined by a stronger negative relationship between age and scores on Letter Number Sequencing test and the Rey Auditory Verbal Learning test compared to visual memory, shown by scores on the Spatial Working Memory test and the Rey Complex Figure Test.
- 3) Furthermore, it was hypothesised, based on the gender differences in serotonin turnover rate, that gender would moderate the effect of the 5-*HTTLPR* genotype on age and memory performance. More specifically, as females have lower levels of serotonin in the CNS, it was predicted that females who carry the s allele would have a greater decline in memory performance with age compared to l-homozygous females and males with either genotype.

## **Methods**

### **Participants**

The study sample consisted of 438 adults aged 50–79 years who had provided informed consent (Appendix A). The mean age of the sample was 60.35 years ( $SD=6.75$ ) and was comprised of 298 female and 140 male participants. All participants were enrolled in the Tasmanian Healthy Brain Project (THBP), a longitudinal study examining the beneficial effects of later life tertiary education on

cognitive reserve, age-related cognitive decline and dementia (Summers et al., 2013). As part of the enrollment process participants were excluded for the following to avoid conditions that may be associated with cognitive impairment: dementia, multiple sclerosis, prior head injury, epilepsy, cerebrovascular complications such as stroke, aneurysm, poorly controlled diabetes, poorly controlled hypertension or hypotension, other neurological disorders, chronic obstructive pulmonary disease, heart disease, blindness or deafness and current psychological diagnosis.

## **Materials**

### **Demographic measures**

To ensure approximate match of genotype groups, information on age, intelligence and previous years of education was collected as these factors have been found to affect performance on the memory tests utilized in the current study (Lezak, 2012; Schoenberg et al., 2006).

The Wechsler Test of Adult Reading (WTAR), a measure of full scale IQ (Strauss et al., 2006) was collected and compared across genotype groups to determine if premorbid intellectual capacity would affect results. The WTAR has established reliability and validity (Green et al., 2008).

The Hospital Anxiety and Depression Scale (HADS; Snaith & Zigmond, 1994) was employed to assess the presence of depression and anxiety symptoms. As depression and anxiety are associated with disruptions of serotonin levels, the HADS self-report scores were compared across genotypes as this could impact cognitive performance (McClintock, Husain, Greer, & Cullum, 2010). HADS is a reliable and valid scale for assessing depression and anxiety with a mean Chronbach's alpha level of .82 for HADS anxiety and .83 for HADS depression scale (Bjelland, Dahl, Haug, & Neckelmann, 2002).

### **Verbal working memory**

The Letter-Number-Sequencing (LNS) Test, a subtest of the WAIS-IV (Wechsler, 2008), was used to assess verbal working memory capacity (Lezak, 2012; Strauss, 2006). This task required participants to mentally rearrange a series of random letters and numbers that were presented to them verbally and then repeat the letters back in alphabetical order and the numbers in order from lowest to highest. The dependent variable (DV) was recorded as the overall number of sequences correctly recalled. The LNS test has excellent reliability and validity (Lezak, 2012; Strauss, 2006)

### **Visual working memory**

The Spatial Working Memory (SWM) subtest of the (CANTAB; Cambridge Cognition Ltd., 2004) assesses visual working memory. Participants were required to search through randomly arrayed boxes and locate coloured tokens within the boxes in order to fill a column on the side of the display. The number of boxes presented is successively increased to alter task difficulty. Total errors in choosing the same box more than once were recorded to comprise the DV. The SWM test has established reliability and validity for older adults (Robbins et al., 1994)

### **Verbal episodic memory**

The Rey Auditory Verbal Learning Test (RAVLT) was used to assess verbal episodic memory (Strauss, 2006). The RAVLT is a 15 word serial list-learning task. There were two lists of words, the first list (list A) was presented across five trials. *Immediate recall:* Participants were required to immediately recall the words presented from list A after each trial. The total amount of words recalled for each trial was recorded as a measure of immediate recall and comprised the DV.

*Interference list:* An interference list (list B) comprising 15 words was presented after the 5 trials of list A had been presented.

*Delayed recall:* Following the recall of list B the initial 15 words from list A were presented again to participants. The total number of words correctly recalled was recorded as a measure of delayed recall and used as a second DV for verbal episodic memory. The RAVLT has established validity for healthy older adults (Strauss, 2006).

### **Visual episodic memory**

The Rey Complex Figure Test (RCFT; Rey, 1941), was used to examine visual episodic memory (Strauss et al., 2006). Participants were required to first copy a complex geometric design on a separate piece of paper. The original design and the copy of the design were then removed from the participants view. After 5 minutes participants were required to re-draw the complex design again from memory. Participants were not informed this would happen to avoid them engaging in retention strategies. Scoring was based on the accurate placement and reproduction of 18 specific elements of the complex design, with higher scores indicating greater performance.

## **Procedure**

### **Neuropsychological testing**

Ethics approval was obtained from the Tasmanian Social Sciences Human Research Ethic Committee (see Appendix B; H11070). The cognitive measures analyzed in this project were part of a larger THBP assessment battery (see Table 2). The memory tests used in the current study were chosen from the battery based on their common use throughout the literature and the specific aspects of memory that each test utilizes (Lezak, 2012). All cognitive tests were administered individually to

each participant by a trained examiner following standard THBP protocol.

Participants underwent baseline testing and follow up testing every two years after baseline (Summers et al., 2013). Due to sample size limitations, only baseline cross-sectional data is presented in the current study. Participants provided saliva samples via Oragene DNA self-collection kits (Genotek, ON, Canada, 2012) that were posted with instructions, information and consent forms (Appendix C). Some participant's attended the medical science precinct in person to have their saliva samples collected after providing informed consent.

### **Genotyping**

Once participants provided saliva samples and their consent the DNA was extracted, purified and then genotyped for tri-allelic variants of the 5-*HTTLPR* (s/s, s/l and l/l). All samples were genotyped in duplicate to ensure accuracy. The 5-*HTTLPR* was identified following a protocol developed by Taylor et al. (2006). The Polymerase Chain Reaction (PCR) mixture contained the forward primer (stpr5, 5'-GGC GTT GCC GCT CTG AAT GC) and reverse primer (stpr3, 5'-GAG GGA CTG AGC TGG ACA ACC AC) which yielded 484-bp (short) and 527-bp (long) fragments. Nine µl of the PCR mixture (see Table D1) was added with 1µl of DNA from the extracted and purified saliva sample in a 96 well plate. One well contained 1µl of DEPC water in replacement of DNA, therefore acting as a no template control. Having a no template control provides a means for checking that there is no contamination. The samples were sealed in the well plate using a Microseal B PCR seal before being run in the PCR thermal-cycler for amplification of DNA. Details of the PCR run are as follows: Denaturation at 94°C for three minutes, 94°C for 30 seconds, annealing at 68°C for 30 seconds, extension at 72°C for one minute for 35 cycles, followed by 72°C for two minutes.

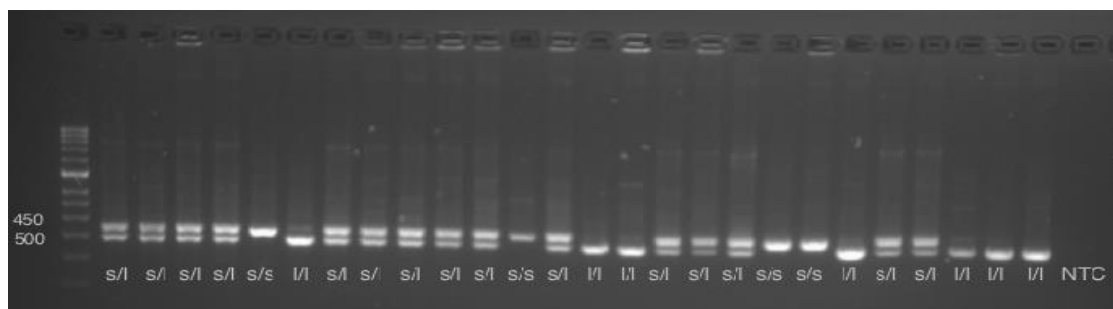
Table 2

*The Tasmanian Healthy Brain Project Test Battery (Summers et al., 2013)*

<b>COGNITIVE RESERVE</b>
Premorbid cognitive reserve
WTAR (Wechsler Test of Adult Reading)
LEQ (Lifetime Experience Questionnaire)
Current cognitive reserve
WAIS-III-SF1 (WAIS-III, short-form)
WRAT4-PMV (Wide Range Achievement Test 4th edition, Progress Monitoring Version)
<b>COGNITIVE/NEUROPSYCHOLOGICAL</b>
Global
DRS-2 (Mattis Dementia Rating Scale, 2nd edition)
Memory
PAL (Paired Associates Learning Test, CANTAB)
RAVLT (Rey Auditory Verbal Learning Test)
LM (Logical Memory Test, WMS-III)
RCFT (Rey Complex Figure Test)
Working memory
SSP (Spatial Span test, CANTAB)
DSP (Digit Span test, WAIS-III)
SWM (Spatial Working Memory test, CANTAB)
LNS (Letter–Number Sequencing test, WAIS-III)
Language
VOC (Vocabulary test, WAIS-III)
COM (Comprehension test, WAIS-III) BNT (Boston Naming Test)
Executive function
COWAT (Controlled Oral Word Association Test)
RVP (Rapid Visual Processing test, CANTAB)
MTS (Match to Sample Visual Search test, CANTAB)
SRT (Simple Reaction Time test, CANTAB)
CRT (5-Choice Reaction Time test, CANTAB)
STROOP (24-item Victoria version Stroop Colour-Word Test)
TMT (Trail Making Test)
<b>PSYCHOSOCIAL</b>
HADS (Hospital Anxiety and Depression Scale)
PWI (Personal Wellbeing Index)
LSNS-18 (Lubben Social Network Scale)
<b>CONFOUNDERS</b>
Medical health status questionnaire
WAIS-III = Wechsler Adult Intelligence Scale, 3rd edition; CANTAB = Cambridge Automated Neuropsychological Test Assessment Battery.

### Gel electrophoresis

Following DNA amplification, the PCR products were run on 2% agarose gel in 1xTAE to visualize the product. The 2% agarose was made using 4g of agarose power and 200ml of 1xTAE. The agarose solution was heated for five minutes in a standard microwave. Gel electrophoresis was run in lots of 30 samples, 75ml of the agarose molten solution was combined with 4µl of Sybr-safe stain. The mix was then poured into a gel casting chamber with a 30 well gel comb to create wells for each sample to be loaded into. Once the gel set, it was placed into a bio-rad gel tank and covered with 1XTAE buffer solution before removing the comb. Five µl of a 100bp marker called DNA HyperLadder V was loaded into the first well, each of the remaining wells were loaded with 9µl of the PCR mixture containing the DNA samples. The gel was then run at 140V for 40 minutes, this allows the samples to migrate through the gel with shorter fragments moving faster than longer fragments and therefore moving further down the gel. Once electrophoresis was complete it was placed on the Image Station that exposes the gel to UV light and using Carestream Molecular Imaging Software the image was captured. Comparing each band to the marker the 5-HTTLPR allele length was determined. A base pair (bp) length of 484 indicated a long allele, 572 indicated a short allele and a band with both bp lengths indicated a heterozygote (see Figure 4).



*Figure 4.* Gel electrophoresis of 26 participant samples and a NTC, the marker is the first ladder on the left used to indicate bp length of DNA fragments.

## Design and Data Analysis

The effect of the *5-HTTLPR* genotype and sex on the relationship between age and memory performance was examined using a moderated moderation cross-sectional design (Hayes, 2013). The independent effects of the *5-HTTLPR* genotype, sex and age on memory function were examined as well as the interaction between these independent variables via moderation analysis. The dependent variables comprised of verbal and visual measures of both working memory and episodic memory were examined in separate moderation analyses. The homozygous s/s and heterozygous s/l genotypes were combined to represent carriers of the short allele and compared against the homozygous l/l genotype. This bi-allelic combination is common throughout the literature as the s allele has a dominant action (Lesch et al., 1996). The bi-allelic approach was also adopted to enhance sample size and statistical power. The two groups were dummy coded (1= s/s and s/l, 2=l/l).

Prior to examining the data for moderation effects, analyses were conducted to determine if there were differences between the two genotype groups that could be considered confounds. Examination of differences between groups for years of education, intelligence and HADS depression score were examined using one-way analysis of variance (ANOVAs). A chi-square goodness of fit test was also used to compare observed genotype frequencies with the expected genotype frequencies for an Australian population to determine if the sample was statistically significantly different from the Hardy-Weinberg equilibrium. Raw scores for the tests of episodic and working memory measures were used for all analyses as examining the effect of age on these scores, made z-scores unnecessary.

All statistical analyses were conducted using SPSS version 22 and the SPSS macro PROCESS (Hayes, 2013) was used to conduct moderated moderation



analyses for each outcome variable. Alpha levels were set at  $p < .05$  for all analyses.  $R^2$  and  $\Delta R^2$  was used as a measure of effect size as other effect size measures could not be computed due to use of categorical predictors (Cohen, 1988). Moderated moderation analysis examines simple moderation (see Figure 5) and the effect of another moderator on the simple moderation (see Figure 6). In simple moderation the moderating variable (M) affects the direction or the strength of the relationship between X and Y (Field, 2013). Identifying a moderator helps to establish the circumstances for which the effect of X on Y is present versus absent, large versus small or positive versus negative (Hayes, 2013).

For all moderated moderation analyses conducted, *5-HTTLPR* genotype was the moderator (M) on the relationship between age (X) and memory outcome measures (Y). The moderator of the moderation was sex (W) for all analyses.

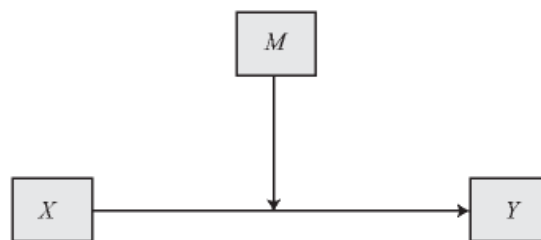


Figure 5. Simple moderation as a conceptual diagram (Hayes, 2013)

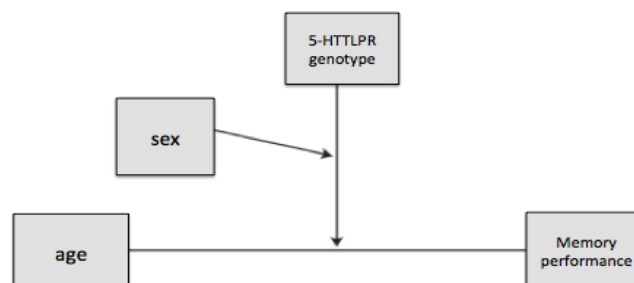


Figure 6. Predicted moderation of sex and *5-HTTLPR* genotype on the relationship between age and memory performance.

## Results

### Assumptions

Prior to regression analysis all variables were examined for the necessary assumptions. Normal probability plots were conducted to examine the assumptions of linearity and homoscedasticity. Scatterplots showed random and even distribution of residuals indicating the assumptions were met. The assumption of normality was examined with frequency distribution histograms and residual scatterplots, indicating normal distributions for all variables. However as suggested by Tabachnick and Fidell (2007) the large sample size and use of moderated regression analyses make this data robust to violations of the normality assumption.

Mahalanobis, Cooks distance and tests of Leverage were employed to detect multivariate outliers in the data set. Using a criterion of  $p < .001$ , cut off scores were: 16.27 for Mahalanobis distance, Cooks distance ( $>.009$ ) and Leverage ( $>.018$ ). Data that met the cut off for two of these tests were considered to be influential and were therefore removed from the data set (Tabachnick & Fidell, 2007: see Table 3).

Collinearity statistics revealed there was no issues of multicollinearity as in accordance with Field (2013) all variance inflation factors (VIFs) were well below ten (highest VIF = 1.01) and all tolerance statistics were above .02 (lowest tolerance = 1). Despite this, variables were mean-centred in moderation analyses which overcome effects of multicollinearity and aid in interpretation of beta coefficients (Field, 2013).

Table 3

*Number of outliers deleted for each outcome measure and the remaining sample size*

Outcome Measure	Outliers Removed	<i>N</i>
RAVLT		
Immediate recall	7	431
Delayed recall	2	436
RCFT Recall	2	435
SWM	1	437
LNS	4	434

*Note.* RAVLT= Rey Auditory Verbal Learning Test, RCFT= Rey Complex Figure Test, SWM= Spatial Working Memory, LNS = Letter Number Sequencing, N = number of participant's.

### **Demographic results and allele frequency distribution**

One-Way ANOVA's were used to test for differences between genotype groups on demographic variables to identify any variables that needed to be controlled for in the moderation analyses. Results revealed no significant differences between the two genotype groups on age, sex, intelligence or years of education. There was also no significant difference between the genotype groups on HADS depression and anxiety scale, prior education or WTAR estimated pre-morbid intelligence. Therefore these variables were not controlled for in any moderation analyses (see Table 4).

A chi-square goodness of fit analysis revealed the distribution of the 5-*HTTLPR* genotype ( $\chi^2_{(2, N=438)} = .058, p = .97$ ), did not deviate significantly from the Hardy-Weinberg equilibrium and aligned with frequencies observed for other Caucasian samples (Caspi et al., 2003; Kendler et al., 2005: see Table 5).

Table 4

*Difference Between 5-HTTLPR Genotypes on Possible Covariates*

	S/S and S/L ( <i>n</i> = 331)	L/L ( <i>n</i> = 107)	<i>P</i>
	M(SD)	M(SD)	
Age	60.25 (6.71)	60.65 (6.90)	.59
Sex			.78
Female	67.67%	69.16%	
Male	32.33%	30.84%	
HADS depression scale	2.45 (2.23)	2.32 (2.18)	.60
HADS anxiety scale	5.29(3.04)	5.23(2.91)	.85
Education (years)	14.08(2.69)	13.51(2.72)	.06
WTAR estimated FSIQ	112.18(5.86)	112.85(5.10)	.29

*Note:* HADS = Hospital Anxiety and depression scale, WTAR = Wechsler Test of Adult Reading, FSIQ = Full Scale Intelligence Quotient.

Table 5

*5-HTTLPR Genotype Frequency Distribution*

Genotype	Observed <i>n</i>	%	Expected <i>n</i>	%
s/s	111	25.2	113.03	25.81
s/l	220	50.2	218.94	49.99
l/l	107	24.4	106.03	24.21

## Moderation Analyses

Moderated moderation analyses were conducted using PROCESS for each outcome variable (RAVLT -immediate and delayed recall, RCFT, SWM test, LNS test). Age, Sex and *5-HTTLPR* genotype were included as predictors in all moderation analyses.

### Episodic memory performance

#### *RAVLT Test Performance*

PROCESS was used to fit linear regression models to test the impact of the *5-HTT* genotype, age and sex on verbal episodic memory performance as measured by RAVLT. The RAVLT provided two measures of verbal episodic memory performance (immediate recall and delayed recall). For both RAVLT outcome measures, age, gender and *5-HTT* genotype were entered as predictors and significant models were produced: total immediate recall ( $F_{(7,423)}=13.77, p<.001, R^2=.18$ ), delayed recall ( $F_{(7,428)}=5.88, p<.001, R^2=.10$ ).

No significant independent association of *5-HTT* genotype on any RAVLT score was found ( $p>.05$ ). Age and sex had a significant negative association with both episodic outcome measures at  $p<.001$ . The independent contribution of each variable in the overall model for episodic memory outcome variables is summarised in Table 6 and Table 7.

No two-way interaction terms were significant, however the three-way interaction (*5-HTT* genotype x age x sex) significantly contributed to the overall model for total immediate recall score,  $\Delta R^2 = .011, p = .016^1$

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<sup>1</sup> Applying Bonferroni adjusted p-values for multiple comparisons, reduced the significance of the three way interaction for immediate recall scores, to trend level, however this adjustment is not commonly used in moderated regression analyses (Myers, 2010) and due to the exploratory nature of the current study, the conservative Bonferroni adjustment was not warranted to reduce the probability of a type two error (Perneger, 1998).

Table 6

*Prediction of RAVLT Immediate Recall Scores From Age, Sex and 5-HTT genotype*

Episodic memory	Predictor	<i>B</i>	<i>SE</i>	<i>T</i>	<i>P</i>	95% C.I	
						Lower	Upper
RAVLT total immediate recall	Age	-.35	.06	-6.18	<b>&lt;.001</b>	-.47	-.24
	5-HTT	.63	.89	.70	.482	-1.13	2.39
	Sex	-4.81	.86	-5.58	<b>&lt;.001</b>	-6.5	-3.12
	Age x 5-HTT	.01	.13	.07	.947	-.25	.26
	Age x Sex	-.12	.13	-.92	.358	-.36	.13
	5-HTT x Sex	-2.5	2.0	-1.23	.221	-6.38	1.48
	Age x 5-HTT x sex	.69	.29	2.42	<b>.016</b>	.13	1.25

Table 7

*Prediction of RAVLT delayed recall score from age, sex and 5-HTT gene*

Episodic memory	Predictor	<i>B</i>	<i>SE</i>	<i>t</i>	<i>P</i>	95% C.I	
						Lower	Upper
RAVLT delayed recall	Age	-.08	.02	-4.08	<b>&lt;.001</b>	-.12	-.04
	5-HTT	.35	.29	1.20	.231	-.22	.91
	Sex	-1.23	.28	-4.34	<b>&lt;.001</b>	-1.79	-.67
	Age x 5-HTT	.01	.04	.25	.802	-.07	.09
	Age x Sex	-.06	.04	-1.38	.169	-.15	.03
	5-HTT x Sex	-.29	.66	-.45	.654	-1.59	1.00
	Age x 5-HTT x sex	.16	.09	1.79	.074	-.02	.34

Simple slopes analysis was conducted in order to break down the three-way interaction for RAVLT total immediate recall scores and to determine if the slopes were significantly different from zero for both males and females separately across the two genotype groups (see Figure 7). These analyses indicated that for males with the long allele there was no significant relationship between age and verbal memory ( $\beta = -.06$ , 95% CI[-.47, .34]  $t = -.31$ ,  $SE = .21$ ,  $p = .75$ ), whereas for males with the short allele, there was a significant negative relationship between age and verbal memory ( $\beta = -.55$ , 95% CI[-.78, -.31]  $t = -4.47$ ,  $SE = .12$ ,  $p < .001$ ). The negative relationship between age and immediate recall score was smaller for females with the short allele ( $\beta = -.27$ , 95% CI[-.42, -.11]  $t = -3.36$ ,  $SE = .08$ ,  $p = .001$ ) compared to females with the long allele genotype ( $\beta = -.48$ , 95% CI[-.73, -.22]  $t = -3.68$ ,  $SE = .13$ ,  $p < .001$ ).

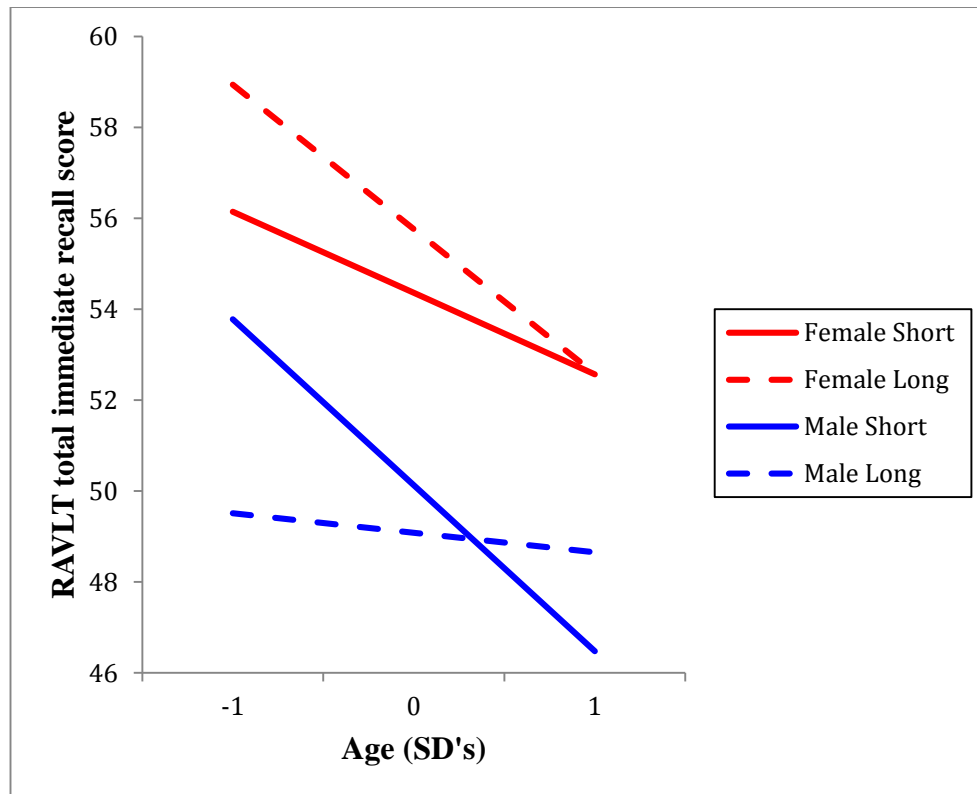


Figure 7. RAVLT total immediate recall scores across age for female 5-HTT genotype groups and male 5-HTT genotype groups.

Figure 7 indicates that females performance on RAVLT total immediate recall test declines across age for both 5-HTTLPR genotypes, however the figure also indicates that the decline in performance is steeper for females homozygous for the long allele compared to female carriers of the short allele. Figure 7 indicates greater decline in immediate recall score for males with the short 5-HTTLPR genotype compared to males with the long genotype.

#### ***RCFT test performance***

The overall model for the RCFT recall was significant ( $F_{(7,427)}=7.82, p<.001, R^2=.12$ ). There was no significant main effect of the 5-HTT genotype, however there was a significant main effect of sex ( $B= 2.07, p<.001$ ) and age ( $B= -.27, p<.001$ ) on RCFT performance, whereby age was associated with a significant decline in RCFT



recall performance. There were no significant two or three way interactions between sex, age and 5HTT genotype on recall for the RCFT (see Table 8).

Table 8

*Prediction of RCFT Recall Score From Age, Sex and 5-HTT Genotype*

Visual episodic memory	Predictor	<i>B</i>	<i>SE</i>	<i>T</i>	<i>P</i>	95% C.I	
						Lower	Upper
RCFT 5 minute recall	Age	-.27	.04	-6.64	<b>&lt;.001</b>	-.35	-.19
	5-HTT	.14	.63	.22	.824	-1.09	1.37
	Sex	2.07	.58	3.59	<b>&lt;.001</b>	.94	3.21
	Age x 5-HTT	-.10	.10	-1.10	.273	-.29	.08
	Age x Sex	.06	.08	.79	.428	-.10	.22
	5-HTT x Sex	-1.54	1.28	-1.20	.229	-4.06	.98
	Age x 5-HTT x sex	.014	.19	.08	.939	-.36	.38

## Working Memory Performance

### *LNS test performance*

For LNS test score the overall model was significant ( $F_{(7,426)}=5.34, p<.001, R^2=.08$ ). There was no significant main effect of the 5-HTT genotype or sex found for LNS performance however there was a significant main effect of age ( $B= -.09, p<.001$ ), whereby LNS test performance declined with age. There were no significant interactions between age, sex and 5-HTT genotype on LNS test performance (see Table 9).

Table 9

*Prediction of LNS Test Score From Age, Sex and 5-HTT Genotype*

Working memory	Predictor	<i>B</i>	<i>SE</i>	<i>t</i>	<i>p</i>	95% C.I	
						Lower	Upper
<b>LNS</b>	Age	-.09	.02	-5.56	<b>&lt;.001</b>	-.13	-.06
	5-HTT	-.03	.26	-.11	.913	-.50	.45
	Sex	.02	.25	.08	.933	-.47	.51
	Age x 5-HTT	-.03	.04	-.63	.532	-.09	.05
	Age x Sex	-.02	.04	-.62	.536	-.09	.05
	5-HTT x Sex	-.16	.57	-.29	.774	-1.28	.95
	Age x 5-HTT x sex	.10	.08	1.31	.191	-.05	.26

### *SWM test performance*

The overall model for SWM test performance was significant ( $F_{(7,429)}=8.21$ ,  $p<.001$ ,  $R^2=.08$ ). There was no significant main effect of 5-HTT genotype or any age, sex, 5-HTT interaction terms. There was however a main effect of age ( $B=.75$ ,  $p<.001$ ) and sex ( $B= -4.3$ ,  $p=.02$ ) on SWM score. Age was associated with a significant increase in errors on the SWM test (see Table 10).

Table 10

*Prediction of SWM Score From Age, Sex and 5-HTT Genotype*

Working memory	Predictor	<i>B</i>	<i>SE</i>	<i>T</i>	<i>P</i>	95% C.I	
						Lower	Upper
SWM	Age	.75	.14	5.46	<b>&lt;.001</b>	.48	1.01
	5-HTT	.60	1.96	.31	.760	-3.25	4.45
	Sex	-4.3	1.89	-2.28	<b>.023</b>	-8.02	-.60
	Age x 5-HTT	.32	.28	1.13	.260	-.23	.86
	Age x Sex	.52	.29	-1.84	.067	-.04	1.08
	5-HTT x Sex	-4.55	4.11	-1.11	.269	-12.64	3.53
	Age x 5-HTT x sex	.53	.58	.91	.361	-.61	1.67

**Discussion**

The present study was designed to explore the differential effects of the 5-*HTTLPR* genotype on the relationship between age and memory performance, while also examining whether this moderation effect was different for females and males. The domains of memory examined were visual and verbal episodic and working memory. The results of this study supported the hypothesis, that the 5-*HTTLPR* genotype would affect the relationship between age and memory performance for verbal immediate recall. The hypothesis that the effects of the genotype would differ across sex was also supported by the significant Age x Sex x 5-*HTTLPR* genotype interaction found for RAVLT total immediate recall scores. While the overall hypothesis that sex and

genotype would moderate the relationship between age and memory performance was supported for one measure of verbal episodic memory, the direction of the findings for females was not in accordance with the hypothesis that s carriers would demonstrate the greatest decline in memory performance across age. Instead, females with the l-homozygous genotype demonstrated a greater decline in verbal immediate recall as compared to female s carriers. In contrast, male carriers of the short allele had greater decline in immediate recall performance compared to males homozygous for the long allele. These findings suggest there may be sex specific factors that interact with 5-*HTTLPR*.

### **The Moderating Effect of 5-*HTTLPR* and Sex on the Relationship Between Age and Memory**

The results of the current study indicate that the 5-*HTTLPR* genotype and sex moderate the relationship between age and memory, confirming the initial hypothesis. There were no two-way interactions between age, sex or the 5-*HTTLPR* genotype suggesting that all three factors need to be considered when looking at the effect of the 5-*HTTLPR* genotype on memory functioning. This is consistent with the prediction that a differential effect of the serotonin genotype would be more readily detected if examined in conjunction with stressors on the serotonin system. Age and sex were both conceptualised as stressors on the serotonin system as they have been shown to influence the levels of circulating serotonin in the central nervous system (Nishizawa et al., 1997; Rodriguez et al., 2012). This moderating effect was, however, only found to predict memory decline for immediate verbal recall and not any other measures of working memory or visual episodic memory function. This supports the second hypothesis that the effects of serotonin would be specific to verbal rather than visual memory.

These latter null findings are inconsistent with O'Hara et al. (2007), who found the s allele to be associated with lower delayed recall compared to the l/l genotype in elderly. However the null findings are consistent with Payton et al. (2005) who also found no significant overall effect of the *5-HTTLPR* genotype on baseline memory performance for older adults. Payton et al. examined the *5-HTTLPR* genotype in 758 elderly Caucasian adults with a similar age range to the current study (50-85years). Participant's memory was assessed using unstandardized measures.

The sample that Payton et al., (2005) used differed from the current sample, as they did not exclude participants with clinical depression therefore examining a broader spectrum of the population. Furthermore, unlike the current study, Payton and colleagues did not consider sex differences in genotype effects on cognitive decline. Whilst the current findings are consistent with Payton et al. as no overall effect of *5-HTTLPR* on memory in the elderly was found, the current findings diverge when including sex as a moderator, something Payton et al. did not do. While no significant association was found for the *5-HTTLPR* genotype on baseline memory measures, Payton et al., did find another polymorphism of the *SLC6A4* gene that codes for the serotonin transporter (VNTR2 polymorphism) to be associated with cognitive decline. This VNTR2 polymorphism however was not associated with significant differential impairment at baseline levels of memory or other cognitive performance. No effect sizes were reported and means were not available for computation of effect size.

The effect size for the moderating effect of *5-HTTLPR* genotype and sex on immediate recall was small, revealing less than two percent of variance in immediate recall performance was predicted on top of the independent contribution of

predictors. The small effect size found in the current study is not uncommon, as highlighted previously, it is unlikely that a single genotype would have a large effect on a behavioural trait (Franks, 2014; Plomin & McGuffin, 2003). Studies that have found moderate effect sizes for the *5-HTTLPR* genotype on memory performance (Price et al., 2013; Weiss et al., 2014; Zilles et al., 2012) have had small sample sizes and a consequence of underpowered studies is inflated effect size (Grissom, 2012). Even in conjunction with stressors on the serotonin system, the current study has shown that genetic effects on a behavioural trait may still be small. Despite small amounts of variance explained, single genotype studies are still relevant in providing insights into what genotypes may be relevant for inclusion in polygenic interactions that account for larger variations in neurocognitive traits (Ward et al., 2014).

### **The Specificity of the Moderating Effect on Verbal Episodic Memory**

The second hypothesis that s-carriers would be associated with a stronger decline in verbal memory compared to visual memory was partially supported, as the only significant three-way interaction found in the current study was for a verbal episodic memory test. There were no significant moderation effects found for tests of visual memory; SWM test and RCFT. However this hypothesis was not fully supported as there was no significant moderation effect of sex and the *5-HTTLPR* genotype on the relationship between age and other measures of verbal memory such as RAVLT delayed recall scores or the LNS test scores.

These findings are consistent with ATD studies that suggest disruptions to the serotonin system negatively affect verbal episodic memory functions more robustly than visual memory functions (Mendelsohn et al., 2009; Sambeth et al., 2007). Findings are also consistent with MDMA research that shows blockage of

uptake by 5-HTT results in impaired verbal memory performance (Laws & Kokkalis, 2007; Wright, Strong, Gilbert, Shollenbarger, & Lisdahl, 2015). These findings converge with findings of Zilles et al. (2012), who found a differential effect of the *5-HTTLPR* genotype on verbal WM performance, not visual memory performance. However Zilles et al. findings differed to the current study as they did not examine episodic memory. Both studies provide evidence for a selective influence of the serotonin genotype on predicting verbal memory performance however.

The inconsistent findings between the current study and Zilles et al., (2012) for verbal working memory, may be due to the different tests of memory function used and the different age range of participants across studies. Differing levels of complexity between different measures may result in variable levels of neurotransmitter involvement, therefore impacting on the comparison between study results (Miranda, 2007). Furthermore Zilles et al. used a mixed sample of healthy participants, bipolar, schizophrenia and obsessive-compulsive participants. Some of their participants were medicated and this was not controlled for, therefore limiting the ability to generalise and compare to the current study that used a more homogenous healthy sample. The current study used a much larger sample size than Zilles et al. and therefore the current findings are suggested to give greater insight into the relationship between *5-HTTLPR* genotype and memory.

### **Females With the Short Allele Should Have Greater Memory Impairments With Ageing**

The third hypothesis that women with the s allele would display greater memory impairments across age was not confirmed. Whilst a significant three-way interaction was found between *5-HTTLPR* genotype, sex and age for verbal episodic

memory, the specific direction of effects evident from the simple slopes analysis were not consistent with the prediction. Instead it was found that females homozygous for the long genotype had the greatest decline in verbal episodic memory performance, with a beta value of .47 compared to a beta value of .27 for females with the s allele (see Figure 7). This finding is inconsistent with Price et al., (2013), who associated the s allele with worse performance for females not males.

Males, however, showed the expected effect, whereby the short allele was associated with significant decline in immediate recall performance opposed to the long homozygous genotype. Overall this group showed the greatest decline in immediate recall across age (beta = .54). The smallest gradient was found for male non-s carriers, which is consistent with the overall hypothesis.

The findings for males are consistent with Roiser (2007) who found the s allele to be associated with deficits in verbal memory functions when individuals have reduced levels of serotonin. However, the findings for females in the current study do not support the findings of Roiser et al. or O'Hara et al, (2007). Despite this, the immediate recall scores for females below the mean sample age of 60.35 years, is consistent with the literature, as scores were lower than l-homozygous females (refer to figure 7).

### **Potential floor and ceiling effects**

Investigation of the difference between the two genotypes at lower age groups of the sample (below mean sample age of 60.35 years), revealed younger female s-carriers had lower immediate recall performance. Alternatively, males with the long homozygous genotype were associated with lower immediate recall performance at younger ages. The non-significant decline in immediate recall across age for males with the l/l genotype may reflect a potential floor effect in the data.



The initial low scores for males with the l/l genotype could mean there is less capacity for decline. It could also be argued that the l/l genotype for females was associated with a stronger decline in immediate verbal recall across age because these individuals have more room for decline due to the higher performance at lower ages.

### **Individual differences in neural compensation**

Individual differences in the ability for neural compensation could confound the interpretation of differences between the *5-HTTLPR* genotype (Stern, 2012). Regions of the prefrontal cortex that show functional variations across age have been shown to have compensatory mechanisms whereby alternative neural structures are used to counteract performance declines (Cabeza, Anderson, Locantore, & McIntosh, 2002). These findings relate to the concept of cognitive reserve, however, they could also be used to explain why the current study did not detect a differential effect of the *5-HTTLPR* genotype on all verbal memory outcome measures across age (i.e verbal working memory and verbal delayed recall) as other studies have (O'Hara et al., 2007).

Little is known about individual differences in the ability to compensate for reduced cognitive functions via activation of alternative neural pathways, however genetics are suggested to play a role (Pacheco, Beevers, McGeary, & Schnyer, 2012; Ward et al., 2014). The individual differences in such abilities could provide some explanation why females and males differed in regards to what allele was associated with the greatest decline in immediate recall.. Perhaps this ability varies not only at the individual level but also by gender. This would provide an explanation as to why in the current study females and males differed in which genotype was associated with the poorest performance at younger ages and older ages.

### **The effect of oestrogen**

The unexpected association between the l/l genotype and greater decline in immediate recall across age for females may be interpreted in relation to the effect of oestrogen on the serotonin system. Oestrogen has been found to act on a variety of receptors, including serotonin receptors (Barth et al., 2015). As the current study examined a healthy elderly cohort with a minimum age of 49 years, the vast majority of females in the sample would have been post menopausal and therefore have relatively stable, low levels of oestrogen compared to younger females (Barth, Villringer, & Sacher, 2015). The majority of previous research that has associated the s allele with increased risk for depression in the face of stressful life events (Brummett et al., 2008; Eley et al., 2004), and worse memory performance (Price et al., 2013; Weiss et al., 2014) in females have examined younger premenopausal cohorts.

The influence of oestrogen levels on memory has been demonstrated by a study that found verbal working memory was affected in females who did not take hormonal contraceptives, whereas females on hormonal contraceptives (relatively stable levels of oestrogen) were not found to have any verbal memory impairments (Rosenberg & Park, 2002). Oestrogen enhances long-term potentiation, which is involved in the formation of episodic memories and also act on serotonin receptors (Barth et al., 2015). Consequently, lower levels of oestrogen seen in postmenopausal women could account for the differences in the effect of the *5-HTTLPR* genotype on memory between younger and older adults. It is therefore suggested that the stability of female oestrogen levels is a possible confound that may need to be controlled for in future studies.

## Limitations

Potential limitations of the present study need to be considered. The cross sectional nature of the current study limits the ability to assess developmental moderations of sex, age and *5-HTTLPR* genotype on memory performance. Using a cross-sectional design makes it difficult to determine whether changes in RAVLT immediate recall score across males and females with different *5-HTTLPR* genotypes predicts developmental progression or instead is the result of cohort differences between participants at each age group. Future studies that investigate a cohort longitudinally would be beneficial for tracking the supposed effects of memory decline associated with the *5-HTTLPR* status across sex.

In the current study the *5-HTTLPR* genotype was investigated as bi-allelic (s-carriers versus l/l genotype) as opposed to investigating the tri-allelic genotype (s/s versus s/l versus l/l) to ensure sufficient statistical power. However this basic split may have reduced sensitivity to the effects of serotonin, even using the tri-allelic genotype may be considered insufficiently sensitive for investigating a single behavioural trait (Plomin & McGuffin, 2003). There are now more nuanced ways of investigating polymorphisms of the *SLC6A4* gene that codes for the serotonin transporter, yet these require much larger sample sizes for adequate power than was available in the current study. It has been identified that another functional polymorphism, Variable Number of Tandem Repeats (termed STin2 VNTR), which has two common variants (10 and 12 16/17bp repeats), acts variably as a transcription enhancer. This genotype has been associated with a differential effect on the rate of cognitive decline in older adults (Payton et al., 2005). This different genotype group may explain the discordant direction of findings in the current study and the non-significant findings for other memory outcome measures. Therefore

future research should consider the *5-HTTLPR* and the *VNTR2* genotype in the same study.

It is important to consider the ethnic homogeneity that comprised the sample of this study. The current study was largely Caucasian and while it is beneficial to study genotype effects in homozygous samples due to differences in genotype frequencies across ethnic groups, results of the current study should not be generalised to other ethnic groups (Goldman et al., 2010).

In addition to considering the samples ethnicity, some bias in the recruitment of participants for this study may be a potential issue as it is highly likely that a study like the THBP would attract highly motivated individuals who have had previous exposure to University or completed year 12. This potential selection bias would likely impact on generalisation of the findings to other demographic groups. Furthermore, the current sample was selected to be psychologically healthy and individuals with depressed or anxious mood were excluded from the study. Thus, future research needs to examine this question in a more representative sample, including measures of depression, current life stress and previous trauma, as they enhance challenges to the serotonergic system with possible neurocognitive consequences.

It should be noted that inconsistencies within the literature are a distinctive feature of almost all studies investigating the association between a single gene and behavioural traits and inadequate power is the most likely explanation (Bertram & Tanzi, 2004; Payton et al., 2005). It has been suggested that at least 80 per cent power is needed for studies to detect a candidate gene that contributes to 1 per cent of the variance in the specific cognitive ability (Payton et al., 2005). It is therefore not surprising that there are inconsistencies in the literature surrounding the

effects of the *5-HTTLPR* genotype on memory as the majority of studies use sample sizes less than 300. The current study did have a larger sample size than majority of the previous literature around the *5-HTTLPR* genotype.

Most studies do not report the effect sizes of the *5-HTTLPR* genotype on memory in their studies, therefore it is difficult to get an estimate of the effect outside of the underpowered nature of studies. However as highlighted, most single genotype studies only account for relatively small amounts of variance. This was also an issue of the current study, as the additional variance in immediate recall performance that was accounted for by the interaction of age, sex and *5-HTTLPR* genotype was only 1.1 per cent.

There are currently no meta-analyses looking at the combined effects of the *5-HTTLPR* genotype on memory or other forms of cognition, therefore it is suggested that this field would benefit from future meta-analyses combining the inconsistent literature as this would increase power to give a clearer picture on whether the *5-HTTLPR* genotype does differentially effect memory decline (Munafo & Flint, 2004). While meta-analyses should not replace a well-designed powerful study, such knowledge is needed in order to determine if the functional polymorphism of the *SLC6A4* gene is a likely candidate for inclusion in more promising studies that investigate polygenetic effects on cognitive performance and cognitive decline.

## **Implications**

This study found a differential effect of the *5-HTTLPR* genotype on the relationship between age and RAVLT immediate recall performance. Furthermore this effect was found to be different across males and females. However there was only a small contribution of the interaction term age, sex and *5-HTTLPR* genotype

on one memory outcome measure and therefore it cannot be concluded that the 5-*HTTLPR* genotype is a marker for predicting rates of memory decline across age. Although other studies have demonstrated this genotype accounts for some variance in memory performance in older adults (O'Hara et al., 2007; Pacheco et al., 2012), due to the small effect size found in the current study and the higher power obtained, it is suggested that future research should focus not on the independent contribution of the 5-*HTTLPR* genotype, but rather look at it in conjunction with other genes associated with memory. For example, genes such as APOE e4 and BDNF have been shown to interact and predict memory performance in older (Ward et al., 2014) and younger (Richter-Schmidinger et al., 2011) adults. The THBP is a longitudinal study and provides the opportunity for investigation of the polymorphic effect the 5-*HTTLPR* genotype, APOE and BDNF genotypes on memory function over time and within individuals. The current findings contribute to the field by highlighting potential sex differences in genotype effects. Therefore a clear implication for future studies trying to identify genetic makers of cognitive function and decline across age should control for sex.

## **Conclusion**

The aim of the current study was to examine whether 5-*HTTLPR* genotype differentially affected the relationship between age and memory performance and determine if this is moderated by sex. It was hypothesised that s allele carriers (s/s and s/l genotype) would be associated with greater decline in memory performance across age compared to individuals with the l-homozygous genotype. It was also hypothesised that the differential effect would be greater for verbal memory performance compared to visual memory performance and for females compared to males.

The hypotheses were partially supported for one outcome measure, verbal immediate recall performance. However the hypotheses were not supported for any other memory outcome measure examined. These current findings highlight the need for future studies to consider sex as a moderator of the functional consequences of the *5-HTTLPR* genotype and other genes on neurocognition. With the expected small effect sizes found in the current study, it is suggested that future research move away from investigating the *5-HTTLPR* as a candidate gene and instead start including this gene in more promising polymorphic investigations. Future investigations that extend on the current study should investigate a broader range of stressors in a more representative elderly population and include other genes in the investigation.

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## Appendix A

### Summary of ATD studies

Table A1

#### *Summary of ATD effects on Memory Performance*

Study	Participants (mean age)	Measure	Effects of ATD		
			Immediate recall	Delayed recall	Delayed recognition
Episodic memory					
Visual					
Kulz et al. 2007	7 OCD patients (28)	RCFT	No effect	-	-
Porter et al. 2005	16 healthy elderly (74)	Rey Visual design learning test	No effect	No effect	-
Hughes et al. 2003	20 healthy (24)	Rey/Taylor complex figure test	-	No effect	-
Verbal					

Reidel et al. (1999)	27 healthy adults (21)	Visual VLT	No effect	Impaired	Partially impaired
Harrison et al. 2004	13 healthy females (22)	Visual VLT	No effect	Impaired	No effect
Hayward et al. (2005)	24 healthy controls (38) 24 depressed patients	RAVLT	No effect on controls Impaired patients	No effect	No effect
Porter et al. (2005)	17 healthy elderly (74)	RAVLT	Impaired	Impaired	-
Kulz et al. (2007)	7 OCD patients (28)	RAVLT	No effect	-	No effect
Merens et al. (2008)	18 remitted depressed patients	Word learning tests	Impaired	Impaired	-
Helmbold et al. (2013)	18 healthy females (24)	German version of the RAVLT	Impaired	No effect	No effect
<b>Working memory</b>			<b>Effect of ATD</b>		
Verbal Luciana et al. (2001)	19 healthy volunteers (22)	Backwards digit span	No effect		

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Stewart et al. (2002)	32 healthy volunteers high neuroticism and low (22)	Backwards digit span	No effect
Porter et al. (2003)	16 AD patients (74)	Backwards digit span	No effect
Porter et al. (2005)	17 healthy elderly (70)	Backwards digit span	No effect
Spatial Luciana et al. (2001)	19 healthy participants (22)	Spatial working memory	No effect
Porter et al. (2003)	16 AD patients (74)	Spatial working memory	No effect
Harrison et al. (2004)	13 healthy females (22)	Spatial working memory	No effect
Porter et al. (2005)	17 healthy elderly (70)	Spatial working memory	No effect

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*Note:* VLT = Verbal learning test, (-) = not tested

**Appendix B**  
Ethics application approval and amendment

MEMORANDUM

Social Science Ethics Officer  
Private Bag 01 Hobart  
Tasmania 7001 Australia  
Tel: (03) 6226 2764  
Fax: (03) 6226 7148  
Marilyn.Knott@utas.edu.au



HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

**FULL COMMITTEE ETHICS APPLICATION APPROVAL**

25 March 2010

Dr Mathew Summers  
Psychology  
Private Bag 1342  
Launceston

Dear Dr Summers

Ethics Reference: **H11070**

Project Title: **The Tasmanian Healthy Brain Study.**

The Tasmania Social Sciences HREC Ethics Committee approved the above project on 24 March 2010.

Please note that this approval is for four years and is conditional upon receipt of an annual Progress Report. Ethics approval for this project will lapse if a Progress Report is not submitted.

The following conditions apply to this approval. Failure to abide by these conditions may result in suspension or discontinuation of approval.

1. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval, to ensure the project is conducted as approved by the Ethics Committee, and to notify the Committee if any investigators are added to, or cease involvement with, the project.
2. Complaints: If any complaints are received or ethical issues arise during the course of the project, investigators should advise the Executive Officer of the Ethics Committee on 03 6226 7479 or [human.ethics@utas.edu.au](mailto:human.ethics@utas.edu.au).
3. Incidents or adverse effects: Investigators should notify the Ethics Committee immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.

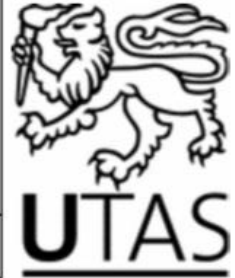


4. Amendments to Project: Modifications to the project must not proceed until approval is obtained from the Ethics Committee. Please submit an Amendment Form (available on our website) to notify the Ethics Committee of the proposed modifications.
5. Annual Report: Continued approval for this project is dependent on the submission of a Progress Report by the anniversary date of your approval. You will be sent a courtesy reminder closer to this date. **Failure to submit a Progress Report will mean that ethics approval for this project will lapse.**
6. Final Report: A Final Report and a copy of any published material arising from the project, either in full or abstract, must be provided at the end of the project.

Yours sincerely

Ethics Executive Officer

Social Science Ethics Officer  
Private Bag 01 Hobart  
Tasmania 7001 Australia  
Tel: (03) 6226 2763  
Fax: (03) 6226 7148  
Katherine.Shaw@utas.edu.au



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HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

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22 December 2011

Dr Mathew Summers  
School of Psychology  
Locked Bag 1342  
Launceston Tasmania

Dear Dr Summers

Re: APPROVAL FOR AMENDMENT TO CURRENT PROJECT  
Ethics Ref: **H0011070 - The Tasmanian Healthy Brain Study**

Amendment Form dated 22 November 2011, revised 13 December 2011.  
Collection of salivary samples from consenting participants for testing of genetic markers associated with elevated risk for dementia.  
Oragene DNA Self-Collection Kit User Instructions.  
Genetic Testing Information Sheet and Consent Form Version 1 dated 2011, revised 21 December 2011.  
Response to NEAF questions regarding genetic testing.

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We are pleased to advise that the Tasmania Social Sciences Human Research Ethics Committee approved the Amendment to the above project on 21 December 2011.

Yours sincerely

Katherine Shaw  
Acting Executive Officer

## **Appendix C**

Information sheets, consent forms and Oragene DNA self-collection kit user  
instructions

## PARTICIPANT INFORMATION SHEET

### Invitation

You are invited to participate in a research study examining the potential benefits of tertiary education in older adults to prevent age-related cognitive decline and dementia.

The study is being conducted by:

- Dr Mathew Summers, Lecturer/Research Fellow, School of Psychology, UTAS;
- Professor James Vickers, Professor of Pathology, School of Medicine, UTAS;
- Dr Nikki Saunders, Research Fellow/Project Coordinator, WDREC

### 1. 'What is the purpose of this study?'

The purpose is to investigate whether older adults undertaking tertiary (University) level education have a reduced rate of age-related cognitive decline and lower rates of dementia than older adults who do not undertake this education.

### 2. 'Why have I been invited to participate in this study?'

You are eligible to participate in this study because you are between 50 and 79 years of age and have no significant health problems that are associated with cognitive impairment.

### 3. 'What does this study involve?'

There are 5 main components to participating in this study that each participant will be asked to undertake:

1. Annual medical health screening questionnaire
2. Annual comprehensive neuropsychological examination
3. Annual psychological health screening
4. Annual assessment of quality of life
5. Completing at least 12 months of University level study at either a full-time or part time load. **Note** participants who volunteer for the control group will not be required to complete any University level study.

The annual medical health questionnaire will ask you to provide details regarding your past and present medical history, current prescribed medication, and use of alcohol, tobacco, and other drugs. In addition, you will be asked to answer questions regarding your age, educational and occupational history, marital status, and family structure.



The comprehensive neuropsychological examination will use a variety of paper and pencil and computerized tests. These tests are designed to provide a detailed examination of a range of specific cognitive functions, including:

- Intellectual ability – as a measure of cognitive reserve
- Memory processing of visual and verbal information
- Short-term memory span
- Working memory capacity
- Language processing
- Visuo-spatial processing
- Higher order executive functioning (attention, concentration, decision making, planning etc).

The psychological health screening involves a brief questionnaire asking you questions regarding your current emotional state; specifically questions relating to experiences of depression or anxiety.

A brief questionnaire will be used to ask you to rate your current quality of life across of range of aspects of your life. For example, how satisfied you are with your relationships with others.

It is expected that the completion of the above assessment will take between 2.5 to 3 hours of your time, including rest breaks. As indicated above, this assessment will occur once every 12 months for up to 10 years and you will be informed individually of your results following each assessment by Dr Summers. Dr Summers is a registered and practicing clinical neuropsychologist and will be responsible for examining the test performances of each participant at each assessment phase. The neuropsychological tests used in this study are clinical tests of cognitive functions, Dr Summers will inform you if you display a clinically significant change to function and you may request such results to be forwarded to your treating medical practitioner if required.

It is important that you understand that your involvement in this study is voluntary. While we would be pleased to have you participate, we respect your right to decline. There will be no consequences to you if you decide not to participate, and this will not affect your treatment / service. If you decide to discontinue participation at any time, you may do so without providing an explanation. All information will be treated in a confidential manner, and your name will not be used in any publication arising out of the research. All of the research will be kept in a locked cabinet in the office of Dr Summers.

#### **4. Are there any possible benefits from participation in this study?**

It is possible that you will notice improved mood and extended social relationships from participating in this study after a certain period of time. This may lead to new opportunities and experiences in your life. We will be interested to see if you experience any other benefits from participating in the Tasmanian Healthy Brain Study.

#### **5. Are there any possible risks from participation in this study?**

With the exception of experiencing some fatigue during the course of the annual assessment, there are no specific risks anticipated with participation in this study. If you experience fatigue during the course of assessment you will be provided with regular rest breaks to reduce this effect. If you find that you are becoming distressed you will be advised to receive support from Dr Mathew Summers or alternatively, we will arrange for you to see a counsellor at no expense to you.

#### **6. What if I have questions about this research?**

If you would like to discuss any aspect of this study please feel free to contact either Dr Mathew Summers on ph 6324 3266 or Professor James Vickers on ph 6226 4830. Either of us would be happy to discuss any aspect of the research with you. Once we have analysed the information we will be mailing / emailing you a summary of our findings. You are welcome to contact us at that time to discuss any issue relating to the research study.

This study has been approved by the Tasmanian Social Science Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study should contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email [human.ethics@utas.edu.au](mailto:human.ethics@utas.edu.au). The Executive Officer is the person nominated to receive complaints from research participants. You will need to quote HREC project number H11070.

**Thank you for taking the time to consider this study.**

**If you wish to take part in it, please sign the attached consent form.**

**This information sheet is for you to keep.**



## **PARTICIPANT INFORMATION SHEET**

### **Genetic Testing in the Tasmanian Healthy Brain Study**

#### **Invitation**

You are invited to provide a sample of saliva for DNA analysis as part of the Tasmanian Healthy Brain Study

The study is being conducted by:

- Dr Mathew Summers, Senior Lecturer/Research Fellow, School of Psychology, UTAS;
- Professor James Vickers, Professor of Pathology, School of Medicine, UTAS;
- Dr Nikki Saunders, Research Fellow/Project Coordinator, WDREC

#### **1. 'What is the purpose of this study?'**

The purpose is to investigate whether genetic markers are associated with the rate of age-related cognitive decline and rate of dementia in older adults, and whether late-life education minimizes the impact of the genetic risk factors.

#### **2. 'Why have I been invited to participate in this study?'**

You are eligible to participate in this study because you are currently participating in the Tasmanian Healthy Brain Study.

#### **3. 'What does this study involve?'**

A donation of a 2 ml sample of saliva, which can be made either in the privacy of your own home or at the Tasmanian Healthy Brain Research centres in Hobart, Launceston or Burnie. Donations made at home can be mailed back to the researchers using prepaid specially designed postal kits.

The DNA samples will be analysed by the Menzies Research Institute of Tasmania. Each sample will only be identified by your unique Tasmanian Healthy Brain Study participant ID code. Only Dr Mathew Summers and Prof James Vickers are authorized to match the participant ID code with your name and contact details.

Unfortunately, we are unable to provide you with the results of the DNA analysis performed. To date there are no known genetic markers that indicate you will develop dementia. At best, there are some genetic markers that appear to be associated with an increased risk for developing dementia, but just as many persons with these genetic markers do not develop dementia as do develop dementia. Therefore, provision of this information to you would be misleading and may result in unwarranted stress and anxiety.



The sample you donate will be stored indefinitely. This will enable researchers to reexamine the DNA you have donated as new genetic markers for different illnesses are uncovered. All DNA samples collected will be destroyed by incineration at the conclusion of the Tasmanian Healthy Brain Study.

In consenting to genetic sampling, you are consenting to not being informed of the results of your genetic testing and to permitting your DNA sample to be retained until the completion of the Tasmanian Healthy Brain Study. If you do not wish to consent to this, you will still be able to participate in the Tasmanian Healthy Brain Study without prejudice. In the *highly unlikely* event that future research identifies a genetic marker that accurately identifies which person will develop dementia with absolute certainty, we will take steps to screen all DNA samples for these markers and provide this information to each individual participant who provides written consent to receiving this information.

#### **4. Are there any possible benefits from participation in this study?**

The DNA sample you provide may assist researchers better understand the role of genetic factors in cognitive function and disease in old age. As such, the benefit from the study may be for future generations rather than an immediate benefit to yourself.

#### **5. Are there any possible risks from participation in this study?**

There are no known risks associated with salivary sample collection.

#### **6. What if I have questions about this research?**

If you would like to discuss any aspect of this study please feel free to contact either Dr Mathew Summers on ph 6324 3266 or Professor James Vickers on ph 6226 4830. Either of us would be happy to discuss any aspect of the research with you.

This study has been approved by the Tasmanian Social Science Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study should contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email [human.ethics@utas.edu.au](mailto:human.ethics@utas.edu.au). The Executive Officer is the person nominated to receive complaints from research participants. You will need to quote **H1070**.

**Thank you for taking the time to consider this study.**

**If you wish to take part in it, please sign the attached consent form.**

**This information sheet is for you to keep.**



## CONSENT FORM FOR GENETIC TESTING

Title of Project: **Tasmanian Health Brain Study – Genetic Testing**

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1. I have read and understood the 'Information Sheet' for this project.
2. The nature and possible effects of the study have been explained to me.
3. I understand that the study involves collection of a 2ml sample of my saliva. I understand I can collect this sample at home and post it back to the researchers or I can donate a sample at the Tasmanian Healthy Brain Study research centres in Hobart, Launceston or Burnie.
4. I consent to not being informed of the results of my genetic testing. I understand that this is because there are no reliable genetic markers for dementia and that the provision of such information would be misleading to me. In the *highly unlikely* event that future research identifies a genetic marker that accurately identifies which person will develop dementia with absolute certainty, I understand that the researchers will screen all DNA samples for these markers and provide this information to each participant who consents to receiving this information.
5. I consent to the storage of my DNA sample until the conclusion of the Tasmanian Healthy Brain Study for use by researchers in the Tasmanian Healthy Brain Study. I understand that my DNA sample will be stored in a secure DNA storage facility at the Menzies Research Institute Tasmania. I understand that at the conclusion of the Tasmanian Healthy Brain Study my DNA sample will be destroyed by incineration.
6. I understand that my DNA sample will not be used by any research other than the Tasmanian Healthy Brain Study.
7. I understand that my DNA sample will be identified only by my unique participant ID code. I understand that only Dr Mathew Summers and Prof James Vickers are able to match my participant ID code with my name and contact details.
8. I understand that all research data will be securely stored on the University of Tasmania premises for at least five years following publication of the results, and will be destroyed when no longer required.
9. Any questions that I have asked have been answered to my satisfaction.
10. I agree that research data gathered from me for the study may be published provided that I cannot be identified as a participant.
11. I understand that the researchers will maintain my identity confidential and that any information I supply to the researcher(s) will be used only for the purposes of the research.
12. I agree to participate in this investigation and understand that I may withdraw at any time without any effect, and if I so wish, may request that any data I have supplied to date be withdrawn from the research.



Name of Participant: \_\_\_\_\_

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

**Statement by Investigator**

☐ I have explained the project & the implications of participation in it to this volunteer and I believe that the consent is informed and that he/she understands the implications of participation

If the Investigator has not had an opportunity to talk to participants prior to them participating, the following must be ticked.

☐ The participant has received the Information Sheet where my details have been provided so participants have the opportunity to contact me prior to consenting to participate in this project.

Name of Investigator \_\_\_\_\_

Signature of Investigator \_\_\_\_\_

Name of investigator \_\_\_\_\_

Signature of investigator \_\_\_\_\_ Date \_\_\_\_\_

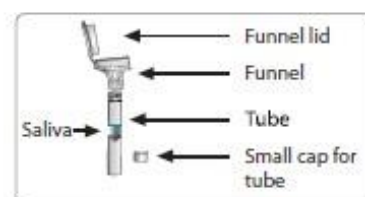







## Oragene®•DNA Self-Collection Kit User Instructions

**Instructions:** Do not eat, drink, smoke or chew gum for 30 minutes before giving your saliva sample.

Do NOT remove the plastic film from the funnel lid.

**Preparation:** Most people take between 2 and 5 minutes to deliver a saliva sample following steps 1 to 5. Before spitting, relax and rub your cheeks gently for 30 seconds to create saliva. If you find it hard to create saliva, place ¼ tsp of white table sugar on your tongue.



- |   |                                                                                     |                                                                                                                                                                                                                                                                 |
|---|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 |    | Spit until the amount of liquid saliva (not bubbles) reaches the fill line shown in picture #1.                                                                                                                                                                 |
| 2 |    | Hold the tube upright with one hand. Close the lid with the other hand (as shown) by firmly pushing the lid until you hear a loud click. The liquid in the lid will be released into the tube to mix with the saliva. Make sure that the lid is closed tightly. |
| 3 |  | Hold the tube upright. Unscrew the tube from the funnel.                                                                                                                                                                                                        |
| 4 |  | Pick up the small cap for the tube. Use the small cap to close the tube tightly.                                                                                                                                                                                |
| 5 |  | Shake the capped tube for 5 seconds. Discard or recycle the funnel.                                                                                                                                                                                             |

**Intended Use:** This product is designed for the safe collection of human saliva samples.

**Warnings:** Wash with water if the liquid comes in contact with eyes or skin. Do not ingest.

**Storage:** Store at room temperature 15-30°C

⚠ **Caution:** Small cap, choking hazard

PD-PR-118 Issue 1.0

**DNAgenotek**  


Manufactured by DNA Genotek Inc.  
 Ottawa, ON, Canada K2K 1L1  
[www.dnagenotek.com](http://www.dnagenotek.com)



## Appendix D

### PCR reaction mixture

Table D1

*PCR Reaction Mixture for Genotyping of 5-HTTLPR Gene*

	Stock concentration	Final concentration	Volume/run (μl)	Volume for 30 samples (μl)
DEPC-treated water		12 μl	3.3	99
MyTaq DNA polymerase	2 X	1 X	5	150
5-HTTLPR forward primer (GGCGTTGCCGCTCTGAATGC)	20 μM	0.05 μM	0.25	7.5
5-HTTLPR reverse primer (GAGGGACTGAGCTGGACAACCAC)	20 μM	0.05 μM	0.25	7.5

